

Temporal Trends in Puget Sound Harbor Seals

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Abstract

Studies of harbor seals provide some of the most consistent data on long-term trends in contamination in Puget Sound. We examined trends in contaminants using samples from harbor seal pups collected at 4- to 5-year intervals from 1972 through the late 1990s in Puget Sound and the Strait of Juan de Fuca. Harbor seals are ideally suited for trend analyses because they are highly contaminated, integrate contamination in a broad selection of prey, and, with proper sample selection, provide low inter-sample variability. We determined levels of a broad range of chlorinated hydrocarbon contaminants including congener-specific concentrations of PCBs, DDTs and other pesticides, and polychlorinated dibenzo dioxins and furans (PCDDs and PCDFs). We tested both the most recent samples and re-tested some of the historical samples from 1984 and 1990 to provide more detailed data on historical contaminants. While concentrations of PCBs and DDT have declined dramatically since the 1970s, concentrations have stabilized since the mid-1980s with only slight declines since then. Even though concentrations have declined, harbor seals are clearly still at risk, with the current concentrations of PCBs and the toxic equivalency quotient (TEQ) in pups within the range identified as causing immunotoxicity in seals. Most of the TEQ came from PCBs rather than PCDDs or PCDFs.

Introduction

Harbor seals (*Phoca vitulina*) are the most abundant marine mammal species in Washington State and occur throughout the marine waters including Puget Sound (Osborne and others 1988). High concentrations of some chlorinated hydrocarbon contaminants, especially PCBs, were found in Puget Sound harbor seals in the 1970s and 1980s (Arndt 1973, Calambokidis and others 1978, 1984). There has been increasing evidence of contaminant-associated adverse effects including reproductive impairment and immunotoxicity and endocrine disruption caused by some chlorinated hydrocarbons in controlled captive feeding studies with PCBs being implicated in the observed effects (Reijnders 1986; Brower and others 1986; Addison 1989; Ross and others 1995, 1996; De Swart and others 1994).

One of the longest-term datasets on trends in contaminants in the Puget Sound region comes from harbor seals. Harbor seal pups from Puget Sound have been collected and tested for concentrations of PCBs and DDT compounds at 4- to 5-year intervals from 1972 to 1990 at several Puget Sound sites (Arndt 1973; Calambokidis and others 1978, 1984, 1991; Hong and others 1996). Samples from harbor seals are ideally suited for trend analyses because they are highly contaminated, represent an integration of concentrations in a broad selection of prey in a region, and, using non-emaciated pups, provide limited inter-sample variability allowing sensitive detection of changes over time.

Primary objectives of the study were:

1. Determine current levels of a broad range of chlorinated hydrocarbon contaminants in Puget Sound harbor seals including congener-specific concentrations of PCBs, DDTs and other pesticides, and the first analyses of polychlorinated dibenzo dioxins and furans (PCDDs and PCDFs).
2. Determine trends in concentrations of some of these contaminants including long-term trends in PCBs and DDT compounds in blubber of harbor seal pups in southern Puget Sound.
3. Determine how concentrations in blubber vary between biopsies of weaned seal pups and those from dead neonates.

4. Identify the degree of inter-sample variability and potential factors responsible for variation (date, length, weight, etc.) in samples from pups and evaluate use of weaned pups in future trend analyses.

METHODS

Sample Collection

New analyses were conducted of 57 blubber samples from harbor seals by the Institute for Ocean Sciences (Table 1). Samples analyzed were obtained in two different ways and five different years from 1984 to 1997. Blubber samples from dead harbor seal neonates in southern Puget Sound were obtained from archived collections for 1984 and 1990 and new samples collected in 1996 and 1997. Additionally, biopsy samples of blubber were obtained from weaned harbor seal pups in 1993 and 1996. Biopsy samples from weaned pups have not been used in the past trend analyses but may be a valuable alternate source of samples. For these samples to be suitable for use in the trend analysis, information is needed on the degree of inter-sample variability, factors responsible for variability, and the comparability of these samples to the past analyses on dead harbor seal neonates.

Table 1. Summary of samples analyzed by IOS for this study.

Type	PCBs, PCDFs & PCDDs	Pesticides
Biopsy samples from captured weaned pups		
1993	11	11
1996	17	12
Total weaned pups	28	23
Samples from fresh dead neonates		
1984	10	10
1990	10	10
1996	4	4
1997	5	5
Total neonates	29	29
Total different samples	57	52

Blubber samples for contaminant analysis were collected from live weaned pups at Gertrude Island, southern Puget Sound in 1993 (n=11) and 1996 (n=17) (Tables 1). Seals were captured using an entanglement net deployed from a boat off the haul-out area (Jeffries and others 1993). Seals were physically restrained then weighed and measured prior to sampling. Newly weaned pups were selected for biopsy sampling for contaminant analyses. Samples were taken by 6 mm diameter sterile biopsy punch from an area over the left pelvis, 10 cm down from the midline. The biopsy site was shaved, cleaned with Betadine solution, and rinsed twice with 70% isopropyl alcohol. A local anesthetic (2.0 cc of Lidocaine:Epinephrine solution) was administered subdermally into the biopsy site. Four biopsy punch samples were taken from each animal. Blubber samples were placed in aluminum foil, then into a whirlpak bag, labeled with identification number, date and collection location. Samples were frozen and stored at -20°C prior to shipping to IOS for analysis.

Dead harbor seal neonates were collected for contaminant analysis from the Gertrude Island area and other regions around southern Puget Sound in 1984 (n=10), 1990 (n=10), 1996 (n=4), and 1997 (n=5) (Table 1). Collections in 1984 and 1990 were part of past studies to examine mortality, causes of death, and contaminants in harbor seals (Calambokidis and others 1985, 1991; Steiger and others 1989) and some of these samples have been analyzed for contaminants previously (Calambokidis and others 1991, Hong and others 1996).

Beach searches were conducted regularly to look for dead pups and birth sites. One or more persons walked the haul-out site and surrounding areas. Additional areas were checked by skiff cruises near shore, using binoculars to scan for carcasses. Searches generally began prior to when pups were born and continued through the end of the pupping season. The Northwest Stranding Network provided additional reports of dead harbor seals found in the study region. Samples chosen for analysis from those collected were based on:

- (1) Post-mortem condition of the animal.
- (2) Collection of blubber, liver, and histopathology samples.
- (3) Presence of an adequate blubber layer (indicating the animal was not emaciated).
- (4) The age of the animal (neonate judged to be no more than one week old).

Carcasses determined to be in good condition were either necropsied at the site or placed on ice and brought back for necropsy in the lab.

Animals were weighed, standard length and axillary girth were measured, and the sex was determined. For information on the age of the pup, the presence of an umbilical cord was noted, and described and measured if present, tooth development was described, and the presence of lanugo coat was noted. Blubber thickness was measured over the posterior end of the sternum (xiphoid cartilage) using a ruler; signs of blubber deterioration (gas bubbles or leaching of oil) were noted.

Tissues were generally sampled with stainless steel instruments that were cleaned by initially rinsing with distilled water, then rinsed with methylene chloride, followed by air drying. Blubber was sampled from the mid-ventral region. These samples included the full thickness of the blubber layer. Toxicology samples were stored on ice if collected in the field and then frozen at -20° C. Tissues shipped to the laboratory for analyses were placed in a cooler with ice and delivered directly or were shipped on dry ice.

Analytical Methods

Approximately 0.1 to 0.2 g of blubber was submitted to the analytical laboratory. Blubber samples were homogenized unfrozen and spiked with a mixture of $^{13}\text{C}_{12}$ -labeled PCDFs, PCDDs and PCBs as supplied by Cambridge Isotope Laboratories (Andover, MA). The PCBs mixture contained representative diortho (DO), mono-ortho (MO) and non-ortho (NO) PCB congeners. The samples were dried with sodium sulphate and extracted with 250 ml of dichloromethane (DCM) from a glass column by gravity flow.

Cleanup took place in three stages. In the first step, aliquots were passed through a multi-layer silica column packed with successive layers of silica gel (basic, neutral, acidic, neutral) and eluted with DCM/hexane (1:1). The second cleanup step was via a neutral alumina activated column capped with anhydrous sodium sulphate. The column was washed with hexane followed with 1:1 DCM/hexane elution to recover the analytes of interest. Fractionation of the later mixture was accomplished with an automated high performance liquid chromatography (HPLC) system utilizing a carbon fiber packed column with a 1:12 mixture of activated carbon/filter paper homogenate. Four fractions were collected from the carbon fiber column: Fraction I was eluted with 20 ml of 5% DCM/hexane and contained the DO-PCBs; Fraction-II eluted with 44ml of 50% DCM/hexane contained the MO-PCBs; Fraction-III, eluted with 50 ml of 50% ethyl acetate/benzene contained the NO-PCBs and Fraction-IV, with 60 ml of toluene in a reverse flow direction to collect all the PCDDs/PCDFs. All four fractions collected from the carbon fiber system were concentrated to less than 10 uL and spiked with the corresponding ^{13}C -labeled method performance standards prior to HRGC/HRMS analysis. Details on the extraction and cleanup methodology utilized, preparation of the silica gel, alumina and carbon fiber columns are described elsewhere (Rantalainen and others 1998, MacDonald and others 1997).

Analyses of cleaned up samples for PCDDs, PCDFs, NO- and MO- and DO-PCBs were conducted by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). For all analyses the MS was operated at 10000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). Details on the GC and MS conditions for the PCDDs/PCDFs, MO- and NO-PCBs analyses have been previously reported for the DO-PCBs analysis (Rantalainen and others 1998; MacDonald and others 1997; Ikonomou and others 1998, in press). The concentrations of identified compounds and their minimum detection limits (MDLs) were calculated by the internal standard method using mean relative response factors determined from calibration standard runs, made before and after each batch of samples was run. The criteria for identification and quantification and the quality control measures undertaken for the HRGC/HRMS analysis of all the analytes of interest were based on procedures established in the Environment Canada "River Road" protocol (Environment Canada 1992a, 1992b) for

PCDD/PCDF analysis. The same criteria and quality assurance quality control procedures were also applied to the NO- MO- and DO-PCB analyses.

Data analysis

No adjustments were made to sample values based on recovery factors. Values for the four duplicate samples analyzed were averaged for the data summaries and statistical analyses. Values below detection limits were treated as 0 values. Total PCBs were determined by summing the values of all PCB congeners quantified. Toxic equivalency quotients (TEQ) were calculated by multiplying concentrations of specific compounds by their newly published toxic equivalency factors (TEF) for mammals (Van den Berg and others 1998). Statistical analyses were conducting using the software package SYSTAT.

Results and Discussion

Duplicate samples

Four sets of samples were analyzed in duplicate. These were not true duplicates because the samples were not homogenized prior to splitting. Additionally, one of these was not a true duplicate because the samples were taken from two separate jars that were collected in the field. Overall there was good agreement among the duplicate samples. For total PCBs and DDTs, differences averaged 12% and 5%, respectively. For total PCBs, all differences were less than 10% except for the duplicates taken from different jars. Total PCDDs and PCDFs differed by slightly larger amounts (25-26%) with greatest differences again from the duplicate samples from different jars. Total TEQ (PCBs, PCDDs, and PCDFs) averaged only a 10% difference among samples.

Analysis results

Results of the new analyses are summarized by group in Table 2. For PCB congeners, 173 congeners or groups were tested for and detectable levels were found for 146. Total PCBs (sum of all detectable congeners) ranged from 2.8 to 44.7 ppm (ug/g, wet weight). Di-ortho PCB congeners accounted for 92-98% of the total PCBs detected, mono-ortho PCBs 2-8% and coplanar PCBs 0.005-0.06%. Higher chlorinated congeners accounted for the highest concentrations with hexa-chlorinated biphenyls responsible for 34-67% of the total PCBs.

Table 2. Summary of concentrations (wet weight) found in Puget Sound harbor seals.

Type/Year	n	PCBs ppm	PCDDs ppt	PCDFs ppt	TEQ ppt
Biopsied live weaned pups					
1993	11	9.9	1043	43.6	116
1996	17	14.3	120	10.3	131
All biopsies	28	12.6	483	23.4	125
Dead neonate pups					
1984	10	17.6	112	6.6	156
1990	10	14.2	114	23.5	175
1996	4	11.2	119	19.3	105
1997	5	9.4	59	9.7	66
All neonates	29	14.1	105	15.2	140
All samples	57	13.4	290	19.4	133

Among the dioxins, concentrations of TCDD ranged from not detected (8 samples) to 45 ppt (ng/kg, wet weight). Total PCDD concentrations ranged from 28 to 2,435 ppt (ng/kg, wet weight). The higher values stemmed from elevated levels of OCDD in one run of samples and may be an artifact. For dibenzofurans, concentrations of TCDF ranged from not detected (3 samples) to 37 ppt with total PCDFs ranging from not detected (2 samples) to 192 ppt.

A number of pesticides were detected in all samples. Highest concentrations were found of different DDT products, particularly p,p'-DDE and p,p'-DDT. Other pesticides with detectable concentrations included chlorobenzenes (tri, tetra, penta, and hexa), HCH (alpha, beta, and gamma), aldrin (detected in only one

sample), dieldrin, alpha-endosulfan, methoxychlor, mirex, chlordane (oxy, trans, and cis), nonachlor (cis and trans), heptachlor, and heptachlor epoxide.

Total TEQs for the samples ranged from 41 to 434 (ng/kg, wet weight). On average, 68% of the TEQ came from mono-ortho PCBs, 23% from coplanar (non-ortho) PCBs, 8% from PCDDs, and 2% from PCDFs. PCBs therefore accounted for over 90% of the TEQ. This is similar to the proportion of TEQs from PCBs occurring in harbor seals showing immune dysfunction in response to feeding on herring from the Baltic Sea (Ross and others 1995).

Based on the total TEQs found in southern Puget Sound harbor seals, they appear to be at risk to immunotoxicity. Mean TEQs (converted to lipid weights) in blubber for southern Puget Sound harbor seal pups were 168 ng/kg. This is close to the mean TEQs in captive Baltic harbor seals showing immune dysfunction; initially estimated as 209 ng/kg (Ross and others 1995, 1996) but recently adjusted to 255 ng/kg to account for updated TEF values and the contribution of congeners not analyzed in the older studies (Ross and others, in press). Eight of the pups sampled in Puget Sound had levels at or above the 255 ng/kg mean TEQ of the immune impaired captive seals. These comparisons are complicated slightly by changes in the TEFs used to calculate the TEQ, but these differences should be small. Because even higher concentrations of contaminants would be expected in older animals, the potential for immune system impairment in portions of the seal population are high.

Comparison of PCBs and DDTs with past analyses of duplicate samples

Some of the samples analyzed for the current study were samples that had been analyzed for PCBs and DDT compounds previously by other laboratories using different methods (see Calambokidis and others 1978, 1984, 1991). We evaluated: 1) the comparability of the current results with those reported previously and 2) the appropriateness of pooling the current results with those from the previous studies.

Of the analyses conducted for the current study at the IOS, seven had also been analyzed for PCBs and DDT compounds at The Evergreen State College (TESC) by Cascadia Research personnel (Calambokidis and others 1984, 1991) and six had also been analyzed by an EPA contract lab (Calambokidis and others 1991). Despite the differences in analytical methods, instrumentation, and quantification methods, there was surprisingly good agreement among the varied analyses. Between the IOS results and those from TESC, means for both PCBs and DDT compounds (p,p'DDE and p,p'DDT) varied by less than 10% between the two labs and differences were not significant ($p > 0.05$, paired t-test). Differences were slightly higher between the common samples analyzed by both IOS and the EPA contract lab for PCBs and DDT compounds. Differences in means were about 20% for both PCBs and DDT compounds, but these were still not significantly different ($p > 0.05$, paired t-test).

The similarity in the total PCBs is particularly surprising given the differences in quantification methods for this complex mixture of compounds. For the current analyses, we used the total PCBs computed as a sum of the concentrations for all detected PCB congeners in the IOS analyses. The EPA contract lab was based on matching the sample profiles to a commercial mixture of PCBs and then utilized selected peaks to extrapolate a total concentration. The TESC analyses quantified the concentration of total PCBs as a sum of the concentrations represented by up to 21 peaks that could be eluted from a packed column. The above results indicate that it would be reasonable to compare the results of the current analyses with some of the historical values. This would also allow the pooling of results from these multiple analysis methods to allow an evaluation of longer time series changes.

Some of the results agree and others disagree with those reported by Hong and others (1996) on concentrations and TEQs for PCB congeners from samples of four southern Puget Sound harbor seals also analyzed in the current study. They report mean values of the four samples that we compared to the means of the same four samples in the IOS analyses. Mean values for total PCBs (13.1 vs. 17.4 ug/g) and p,p'-DDE (2.9 vs. 2.3 ug/g) agreed reasonably well as did values for most of the principal PCB congeners. Calculated TEQs from PCBs, however, differed greatly primarily as a result of differences in the concentration of one congener (PCB 126). This coplanar non-ortho PCB has a high TEF (0.1) and contributed over 75% of the total TEQ calculated in Hong and others (1996). The mean concentration of this congener reported by Hong and others (1996) was more than an order of magnitude higher than those found in the current analyses of the same

samples (7.7 vs. 0.54 ng/g). We suspect the higher value reported in Hong and others (1996) may have been biased high by inclusion of some potential co-eluting congeners.

Relationships with year, type of sample, and animal condition

Total PCBs and DDTs showed a decreasing trend but Analysis of Covariance (ANCOVA) revealed no significant differences between biopsied weaned pups and dead neonates or significant decline with year for the 1984-97 samples analyzed at IOS ($p > 0.05$ in both cases). Total TEQ showed a nearly significant decline by year ($p = 0.07$). Other pesticides also showed general declining trends but only for HCB, total chlorobenzenes, and chlordanes were the declines statistically significant.

Examination for trends and differences in condition was also conducted with the inclusion of a number of other factors that appeared to be influencing concentrations of contaminants. Multiple regression analyses incorporating weight and length of the pup generally revealed significant trends in contaminants and some or all of these factors including sometimes improving the significance of the trend by year. For total PCBs, there were significant differences between weaned and dead pups, an inverse relationship with weight and direct relationship with length ($p < 0.05$ in all three cases), while the trend by year fell just short of significant ($p = 0.08$).

Because of the large difference in size between biopsied and dead neonate pups, we also examined patterns just among biopsied seals. Even within this group, concentrations of contaminants varied significantly by size and condition of the animal. Among biopsied pups, concentrations of total PCBs and total TEQ varied significantly directly with length and inversely with weight (multiple regression, $p < 0.01$). For total DDTs the pattern was similar, although the relationship with length fell slightly short of significance ($p = 0.08$). The relationships with length and weight were not as easily discerned with some of the other pesticides and with PCDDs and PCDFs, possibly due to the greater variations in these measurements and the slightly smaller sample size of biopsied animals examined for pesticides.

Inclusion of previous samples from past analyses of total PCBs and DDTs would allow evaluation of a longer time series (back to the 1970s). Earlier samples were primarily analyzed by Cascadia at The Evergreen State College (TESC), and the results with duplicate samples showed good agreement with the total PCB and DDT values determined by IOS. This close agreement among labs suggests pooling these results would not strongly bias the trend analysis. There were no duplicate samples available to test the agreement with a few analyses conducted in the early 1970s at the University of Washington (Arndt 1973). Both total PCBs and DDTs showed highly significant declines by year when the results of previous analyses from the 1970s and 1980s are included.

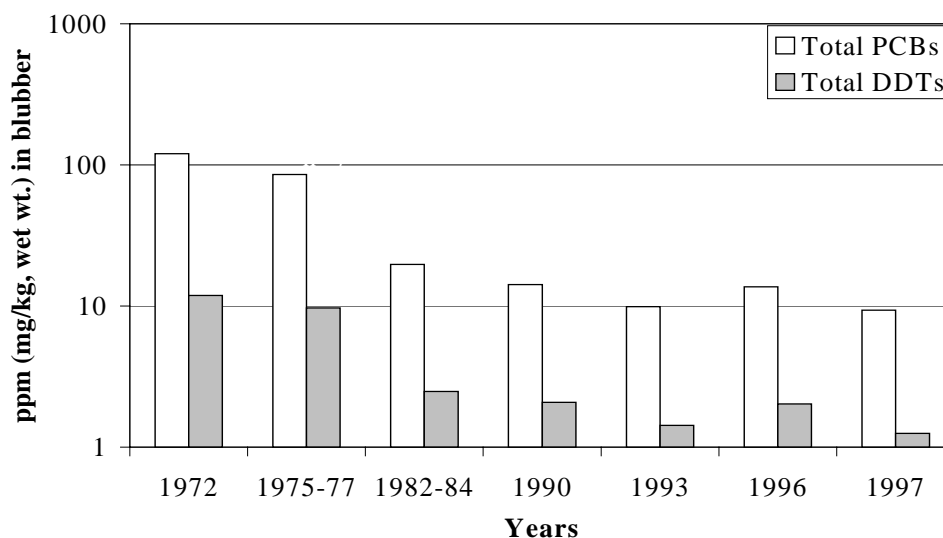


Figure 1. Trends in Total PCBs and DDTs using current and historical datasets (see text).

These results indicate there was clear decline in PCB and DDT concentrations in harbor seals between the 1970s and 1980s but that this decline has slowed and become less pronounced in the 1990s. Although initial efforts to restrict use of these compounds have resulted in a drop in concentrations, these long-lived contaminants persist in the marine environment and high concentrations in some areas such as Puget Sound will likely remain for years to come. Given these concentrations are in the range of those shown to cause immune response in other studies, harbor seals in this region may be at risk for some time to come.

Conclusions

Primary conclusions from this study include:

1. Dead neonates and biopsied live weaned pups yielded similar blubber concentrations.
2. Total PCBs and DDTs showed good agreement with analyses done in the past.
3. Although PCBs and DDTs have declined since the 1970s, levels have stabilized in recent years.
4. Primary risk (measured by TEQs) comes from PCBs and not PCDDs or PCDFs.
5. PCBs and TEQs remain high and similar to those known to cause immune dysfunction.

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